

REMARKS

Applicants respectfully request entry of the above amendments and reconsideration of the following arguments pursuant to 37 C.F.R. § 1.111. The amendments are made without disclaimer or prejudice to Applicants' rights to pursue any canceled subject matter in this or a timely filed continuing application.

1. Status of the Claims

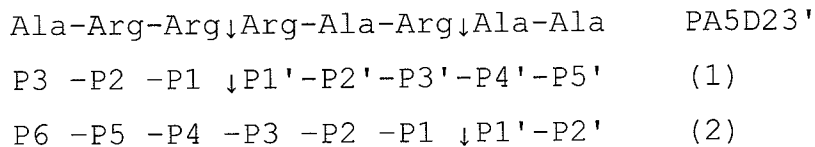
Claims 1-35 are canceled without prejudice or disclaimer. New claims 36-68 are added. The new claims correspond to original claims 1-35, as shown by a side-by-side comparison in **Exhibit 1**. (**Exhibit 1** is *not* a "listing of claims.") All the new claims correspond to claims that are rejected.

2. Support for Amendments

The newly added claims 36-68 clarify several aspects of the invention and are reordered for greater clarity. The subject matter of the new claims is supported at least by claims 1-35, as shown by a side-by-side comparison between the claims in **Exhibit 1**.

In claim 36 (corresponding to claim 1), "steps of" is not recited, and the number of method elements is reduced to a "cleaving" element. Claim 36 adds "a cleavage site that is a peptide bond between a P1 position and a P1' position," which is supported at least by the definition of a "cleavage site" at page 2, lines 14-23, of the specification. Claim 36 also incorporates the elements of claim 4.

Claim 36 is further amended to delete the phrase "or from the P3' position to the P5' position." Support for this amendment is provided throughout the specification, for example, by original claims 10 and 11 and by the sequence PA5D23' in FIG. 4. PA5D23' has two cleavage sites and thus two possible P1/P1' pairs, shown below in sequences (1) and (2):



The PA5D23' sequence contains a basic amino acid (i.e., Arg) at position P3', as shown in formula (1). Likewise, two or three consecutive basic amino acids are situated in the same amino acid sequence from the P10 to P3 positions, as shown in formula (2).

Claim 49 (corresponding to claim 12) is amended to incorporate the subject matter of original claim 13 (*see Exhibit 1*). Claim 49 also adds "a cleavage site that is a peptide bond between a P1 position and a P1' position," which is supported at least by the definition of a "cleavage site" at page 2, lines 14-23.

Claim 54 (corresponding to claims 15 and 16) now depends from claim 49. Claim 54 does not recite the element of "transforming host cells." In some of the claims, for example claim 57 (corresponding to claim 18), "polypeptide or the fusion protein" is substituted with "polypeptide" for greater clarity. Original claim 35, which depended from claim 1 or claim 12, now corresponds to new claims 41 and 56. To clarify antecedent basis, claim 41 depends from claim 37 (which corresponds to claim 2), and claim 56 depends from claim 54 (which corresponds to claim 15).

Support for new claims 67-68 can be found at least from lines 6-11, on page 18 of the Specification (the target peptide may be cleaved by either an *E. coli* OmpT or an *E. coli* OmpT protease 97th amino acid variant).

The new claims thus are believed to be supported by the specification as filed and to add no impermissible new matter.

3. Acceptance of Drawings

Applicants appreciate the Office's acknowledgement that the drawings filed on March 26, 2008, are deemed acceptable.

4. Priority Document

Applicants appreciate the Office's acknowledgement that copies of the certified copies of the priority documents have been received from the International Bureau. The Office notes that a certified translation of JP 2003/342183 is not of record.

5. Acknowledgement of Information Disclosure Statements

Applicants note with appreciation the acknowledgement of the Information Disclosure Statements (IDSs) filed July 24, 2009, and September 14, 2009. The Office is respectfully requested to acknowledge the Search Report dated December 13, 2004, on the IDS submitted November 15, 2007.

6. Prior Office Actions

The Office Actions of February 9, 2009 (Restriction Requirement) and May 14, 2009 are withdrawn.

7. Rejection under 35 U.S.C. § 101

No grounds are provided supporting the rejection under 35 U.S.C. § 101. The rejection thus should be withdrawn. To the extent the rejection is based on allegations made in the context of the following non-statutory obviousness-type double patenting rejection, Applicants respond fully in the next section of the response.

8. Obviousness-Type Double Patenting Rejection

The Office rejects claims 1, 4-7, and 34-35 (corresponding to new claims 36, 43-45, 48, and 41, respectively, as shown in **Exhibit 1**) under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1-3, 12, and 19 of U.S. Patent No. 6,037,145 ("the '145 patent"). The Office alleges that the conflicting claims are not patentably distinct.

The new claims are patentably distinct from claims 1-3, 12, and 19 of the '145 patent for at least the following reasons. Obviousness-type double patenting rejections unsupported by

evidence are reversed. *See Ex parte Whalen*, 89 USPQ2d 1078, 1081 (Bd. Pat. App. & Int. 2008) (precedential). Present claim 36 recites in part:

... wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position.

The '145 patent claims do not recite at least these elements. Claim 19 of the '145 patent recites a linker peptide having the amino acid sequence RLYRRHHRWGRSGSPLRAHE, which is cleaved by OmpT as follows: RLYR↓RHHRWGRSGSPLRAHE. *See* the '145 patent, FIG. 6. The '145 patent sequence thus has the following P and P' positions:

Cleavage Motif	R	L	Y	R	↓	R	H	H	R	W	G	R	S	G	S	P	L	R	A	H	E
Notation of Positions	P4	P3	P2	P1	↓	P1'	P2'	P3'	P4'	P5'											

The '145 patent sequence contains a single Arg at P4. It does not contain two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as presently claimed. The Office fails to provide evidence why present claim 36 is patentably indistinct from claim 19 of the '145 patent in light of this difference. New claims 43-45, 48, and 41 (corresponding to claims 4-7 and 34-35, respectively) depend from claim 36 and incorporate the elements of present claim 36. The dependent claims thus are likewise patentably distinct from the '145 patent claims. The rejection accordingly is unsubstantiated and must be withdrawn. *See Ex parte Whalen*, 89 USPQ2d at 1081 (precedential).

The Office appears to reply in part on embodiments disclosed, but not claimed, in the '145 patent.¹ “[A]n earlier patent’s disclosure is not available to show nonstatutory double patenting.” *See General Foods Corp. v. Studiengesellschaft Kohle mbH*, 972 F.2d 1272, 1281-82, 23 USPQ2d 1839, 1846 (Fed. Cir. 1992). To the extent the rejection relies on the '145 patent disclosure, but not the '145 patent claims, the rejection is improper and must be withdrawn.

¹ “The portion of the specification in US 6,037,145 that supports the recited methods includes embodiments that would *anticipate* Claims 1, 4-7, 34 and 35 herein, e.g., methods for cleaving fusion proteins using an OmpT protease, which are also the methods specifically recited in Claims 1-3, 12, and 19 of US 6,037,145.” Office Action, page 3 (emphasis added).

9. Rejection under 35 U.S.C. § 112, Second Paragraph

The Office variously rejects claims 1-35 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

[A] Claims 1-35 (corresponding to new claims 36-68)

Claims 1-35 are allegedly indefinite for reciting “*E. coli* OmpT protease.” Applicants traverse the rejection as it applies to new claims 36-68.

The Office alleges that neither the specification nor the art at the time of the invention defines the structure and function of *E. coli* OmpT proteases. It is well established that “the definiteness of the language employed must be analyzed-not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art.” *In re Moore*, 169 USPQ 236, 238 (CCPA 1971).

The Office alleges that the skilled artisan would not clearly understand what is meant by an *E. coli* OmpT protease, in part because of the results of a BLAST sequence alignment with GenBank Acc. No. AAA24430.1. The Office specifically refers to an alignment of Acc. No. AAA24430.1 with Acc. No. YP_444072.1. This alignment is attached to the Office Action. The alignment shows that the two sequences have 73% identity.

The GenBank description provided in Acc. No. YP_444072.1 is attached as **Exhibit 2**. In **Exhibit 2**, the skilled artisan discloses that the protein is encoded by an “OmpT” gene. Accordingly, “*E. coli* OmpT protease” is definite, when analyzed as it would be interpreted by the skilled artisan. The skilled artisan clearly understood that the protein of Acc. No. YP_444072.1 was (1) from *E. coli* and (2) an OmpT protease, as seen by the annotation of the sequence in Acc. No. YP_444072.1. The skilled artisan clearly understands which proteins are encompassed by the term “*E. coli* OmpT protease.” Nothing more is needed to comply with 35 U.S.C. § 112, second paragraph. *See Moore*, 169 USPQ at 238.

The Office’s remaining allegations concern uncertainties in the art about OmpT protease substrate specificities. Even if true, for the sake of argument, the allegations have no relevance to whether the skilled artisan would recognize what is meant by an “*E. coli* OmpT protease.” Chemical entities encompassed by a claim do not have to fall within an arbitrary degree of

structural and/or functional similarity to comply with 35 U.S.C. § 112, second paragraph. *See Moore*, 169 USPQ at 238; *cf. In re Borkowski*, 164 USPQ 642 (CCPA 1970). Nor is there a statutory requirement that the artisan needs to know the precise substrate specificities of *E. coli* OmpT proteases. For compliance with 35 U.S.C. § 112, second paragraph, the artisan just has to be able to determine the boundaries of the claim. The artisan can, as seen from the evidence on the record. The rejection thus is unsubstantiated and must be withdrawn.

[B] Claims 1-35 (corresponding to new claims 36-68)

Claims 1-35 are allegedly indefinite for reciting the phrase “desired cleavage site.” The phrase does not occur in the present claims, mooting the rejection. The rejection can be withdrawn.

[C] Claims 2 and 31-33 (corresponding to claims 37 and 38-40)

Claim 2 and 31-33 are allegedly indefinite for reciting “the C-terminus of the protecting peptide is the P1 position and the N-terminus of the protecting peptide is the P1' position.” Corresponding new claims 37, 38-40, and 67-68 use the Examiner’s suggested language. The rejection thus may be withdrawn.

[D] Claims 8-9 and 23

Claims 8-9 and 23 are allegedly indefinite for reciting “producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein.” No new claims correspond to claims 8 and 23. New claim 66 (corresponding to claim 9) does not contain the allegedly indefinite phrase. As the language at issue does not occur in the new claims, the rejection is thus moot. The rejection can be withdrawn.

[E] Claims 14 and 17 (corresponding to claims 51 and 55)

Claims 14 and 17 are allegedly indefinite for reciting “any site.” The Office alleges it is unclear whether “any site” refers to “any position” or “the desired cleavage site.” Office Action, page 7.

The new claims state that a “cleavage site” is “a peptide bond between a P1 position and a P1' position.” In the new claims, “cleavage site” is not used in the context of amino acid

positions P10 – P5', and the language at issue does not occur in the new claims. (Applicants for clarity sometimes refer to positions P10 – P5' in this response as a “cleavage motif.”) Applicants respectfully request withdrawal of the rejection.

[F] Claim 16 (corresponding to claim 54)

Claim 16 is allegedly indefinite for reciting “a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site.” The Office alleges that the clause may refer to (1) a fusion directly between a protecting peptide and a target peptide; (2) a fusion between a protecting peptide and a target peptide, via a linker; and (3) either of the above. Office Action, page 8. The Office apparently interprets “via a desired cleavage site” as “via a linker.”

New claim 54 incorporates the elements of claim 49. Claim 54 thus recites a fusion protein of the structure:

protecting peptide–P1↓P1'–target peptide,
where C-terminal P1 = Arg or Lys, and
where N-terminal P1' ≠ Arg or Lys.

Accordingly, claim 54 is clear, and the rejection should be withdrawn.

[G] Claim 34 (corresponding to claim 48)

Claim 34 is allegedly indefinite for reciting “using, as the cleaving protease, bacterial cells expressing a gene coding for.” The clause is allegedly unclear, because the claim may include using intact bacterial cells, a lysate thereof, an/or the isolated encoded protease. Office Action, page 8.

Applicants traverse the rejection. Applicants can claim what they regard as their invention. 35 U.S.C. § 112, second paragraph. New claims 37 and 54 are directed processes, where the cleaved “polypeptide” is a “fusion protein.” The claims only require that the fusion protein be “produced by expressing a gene encoding the fusion protein in a host cell.” By the plain language of the claims, the claim encompasses processes where the fusion protein is, for example, expressed and cleaved in an intact host cell, expressed and cleaved in a host cell lysate,

or optionally purified. This is not a situation where the claims are indefinite, because claim terms are amenable to two or more plausible claim constructions. *Compare Ex parte Miyazaki*, 89 USPQ2d 1207, 1211 (Bd. Pat. App. & Inter. 2008). Instead, the claims clearly encompass all the constructions at issue. The claims are definite, and the rejection thus must be withdrawn.

[H] Claim 35 (corresponding to claims 41 and 56)

Claim 35 is allegedly indefinite for reciting the term “co-expressed.” It is allegedly unclear whether the term means recombinant co-expression and/or endogenous co-expression. Office Action, page 8. New claims 41 and 56 by the plain claim language clearly encompass both interpretations. The claims are not indefinite for the same reasons as set forth above at Part 9[G]. The rejection thus should be withdrawn.

[I] Claims 2-3, 12, 15-17, 27, and 34-35

(corresponding to claims 37, 42, 49, 54-55, 50, 48, 41, and 56, respectively)

Claims 2-3, 12, 15-17, 27, and 34-35 allegedly lack proper antecedent basis. Applicants traverse the rejection, responding in order to each issue raised in the Office Action:

- Claims 2 and 3: New claim 42 refers to “the *E. coli* OmpT protease” as suggested.
- Claims reciting “97th amino acid from the N-terminus of the OmpT protease” recite “97th amino acid from the N-terminus of the *E. coli* OmpT protease 97th amino acid variant,” as suggested.
- “Desired cleavage site” no longer occurs in the claims, mooted the rejection.
- Claim 17: New claim 55 no longer recites “fusion protein,” mooted the rejection.
- New claim 55 recites “*the* amino acid sequence,” which the Examiner alleges lacks antecedent basis. Applicants do not use the alternative phrase “*an* amino acid sequence,” because this Technology Center typically interprets “an” amino acid as including a single amino acid. Considering the claim as a whole, “the amino acid sequence” clearly refers to all the amino acids situated from the P10 to P3 positions. The rejection thus should be withdrawn.
- Claim 35: New claims 41 and 56 do not recite “a polypeptide,” mooted the rejection.

In summary, the claims comply with 35 U.S.C. § 112, second paragraph. The rejections thus must be withdrawn.

10. Rejection under 35 U.S.C. § 112, First Paragraph (Enablement)

Claims 12-30, 32, 34, and 35 stand rejected under 35 U.S.C. § 112, first paragraph, allegedly as non-enabled. Applicants traverse the rejection as it applies to corresponding the new claims. Both independent claims 36 and 49 are implicated in the rejection, because claims depending from both independent claims are rejected. The new claims at issue are claims 36, 39, 41, 48-65, and 67, as indicated in **Exhibit 1**.

GROUND FOR REJECTION

The Office alleges that the claims are enabled only for specific exemplified combinations of OmpT variants and cleavage motifs. The Office acknowledges numerous examples of operative embodiments described in the specification and guidance available from the state of the art. The Office, however, alleges that these examples do not reasonably correlate with the scope of the claims. The Office emphasizes the scope of the claims encompasses 20¹³ cleavage motif sequences. *See, e.g.*, Office Action, bottom of page 14. The Office also acknowledges that the amount of experimentation to practice the full scope of the claimed invention is not undue, if the experimentation is routine in nature and the techniques necessary to perform the experimentation are well known to the skilled artisan. The Office, however, alleges that the experimentation to test the embodiments commensurate with the scope of the claims is not routine, because of the unpredictability of the art.

ARGUMENT

Whether undue experimentation is required to practice an invention is determined by the *Wands* factors: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *In re Wands*, 858 F.2d 731, 736, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988).

[A] Claim 36 and its dependent claims

Claim 36 as amended is directed to process for cleaving a polypeptide with one of a genus of OmpT cleavage motifs. In the cleavage motifs, the P1 position is arginine or lysine; the P1' position is an amino acid other than aspartic acid, glutamic acid or proline; and two or three consecutive basic amino acids are situated in the amino acid sequence from the P10 position to the P3 position. The claim is directed to “cleaving” polypeptides having this motif. Giving claim 36 its broadest reasonable interpretation consistent with the specification and with the interpretation the skilled artisan would reach (*see In re Cortright*, 165 F.3d 1353, 1358 (Fed. Cir. 1999)), the claim reads on cleaving with any degree of efficiency.

Amended claim 36 encompasses a large number of motifs, although fewer than 20¹³. The working examples in the specification show that OmpT cleaves numerous motifs within the scope of the claims, as the Office acknowledges. The degree of exemplification reasonably correlates with the scope of the claims.

The Office must provide objective evidence or reasoning that the skilled artisan would doubt the enablement provided by the specification. *See In re Cortright*, 165 F.3d 1353, 1357, 49 USPQ2d 1464 (Fed. Cir. 1999). The Office, however, only questions whether all motifs would be cleaved with equal efficiency, not whether OmpT could not cleave the motifs. It is possible that OmpT does not cleave some motifs encompassed by the claims at all. Even in an unpredictable art, however, long established judicial precedent holds that exemplification of each and every embodiment encompassed by a claim is not required to comply with 35 U.S.C. § 112, first paragraph. *See In re Angstadt*, 190 USPQ 214, 218 (CCPA 1976). Applicants provide the degree of exemplification required by law, so the rejection should be withdrawn.

[B] Claim 49 and its dependent claims

The Office alleges that the specification enables only specific combinations of OmpT variants and cleavage motifs that are shown in the examples. Claim 49 is directed to a process comprising cleaving a polypeptide with an OmpT 97th amino acid variant, where the 97th amino acid is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid, or histidine. Further, the variant cleaves at motif, where the P1

position is arginine or lysine, and the P1' position is an amino acid other than arginine or lysine. Claim 49, like claim 36, does not require the OmpT variant to cleave any recited motif with a particular efficiency. The OmpT variants recited in claim 49 cleave at least one recited cleavage with some efficiency. The Office produces no evidence or reasoning why any variant would not cleave another recited motif at all.

The Office alleges that the disclosure of Kramer² provides objective evidence of non-enablement. Referring to Kramer, p. 429, ¶1, and FIGS. 2-4, the Office alleges that Asp⁹⁷ is “critical” for cleavage site recognition and that altering Asp⁹⁷ changes cleavage efficacy. *See* Office Action, p. 13, ¶2. To the contrary, Kramer states (p. 429, 1st col., ¶1):

D97A OmpT displayed only 6% residual activity; therefore, we propose that Asp⁹⁷ is responsible for the observed P1' specificity.

Kramer uses the following substrate (p. 427, 1st col., ¶3):

Abz-Ala-Arg-Arg-Ala-Dap(dnp)-Gly
P2 -P1 ↓P1'-P2',

where Abz is o-aminobenzoyl, and Dap(dnp) is N-β-dinitrophenyl-L-diaminopropionic acid. FIG. 4 of Kramer shows that this substrate is cleaved between the two arginine residues. *See also* Kramer *et al.*, *Eur. J. Biochem.* 267: 885-893 (2000), p. 887, 2nd col., 4th line from the bottom.

Enablement is determined with respect to the claimed invention. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Claim 49 and its dependent claims recite a cleavage motif, where the P1' position is an amino acid other than arginine or lysine. The P1' position of Kramer, however, is arginine. Kramer's disclosure relates to an unclaimed embodiment. Kramer is not evidence that a claimed embodiment is inoperable.

In conclusion, the rejection of claim 49 is unsubstantiated for the same reasons as the rejection of claim 39. The rejection thus should be withdrawn.

² **Exhibit 3** lists abbreviations of the cited references.

11. Rejection under 35 U.S.C. § 112, First Paragraph (Written Description)

Claims 1-3, 6, 7, and 31-35 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The corresponding new claims are claims 36-42, 44-45, 48, and 67-68.

GROUND FOR REJECTION

The Office requires reduction to practice of representative examples of cleavage motifs with each recited amino acid substitution, and that a single reduction to practice is an inadequate description of a particular cleavage motif.

ARGUMENT

A fully described genus must allow one skilled in the art to “visualize or recognize the identity of the members of the genus” and to “distinguish the claimed genus from others.” *University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1568 (Fed. Cir. 1997). “[W]hat is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, [and] the predictability of the aspect at issue.” *Capon v. Eshhar*, 418 F.3d 1349, 1359 (Fed. Cir. 2005). “[I]t is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. See *In re Angstadt*, 537 F.2d 498, 504 (CCPA 1976); *Capon*, 418 F.3d at 1359.

In the present case, claim 39 clearly distinguishes a subgenus of cleavage motifs from the genus of all possible OmpT cleavage motifs. The cleavage motif comprises a cleavage site that is a peptide bond between a P1 position and a P1' position;

- wherein the P1 position is arginine or lysine;
- wherein the P1' position is an amino acid other than aspartic acid, glutamic acid or proline; and

- wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position.

Although this art is unpredictable in nature, the specification shows numerous examples of OmpT cleaving the recited motifs. The examples in the specification show that claim 36 encompasses functional cleavage motifs, providing a reasonable correlation between structure and function. *See, e.g., Enzo Biochem Inc. v. Gen-Probe Inc.*, 296 F.3d 1316, 63 USPQ2d 1609 (Fed. Cir. 2002). From the degree of exemplification, the artisan would understand that Applicants possessed the invention at the filing date.

Claim 36 further does not require a particular efficiency of cleavage. While it is possible that some motifs encompassed by the claims may not be cleaved at all, there is no requirement under relevant U.S. judicial precedent to show reduction to practice of every permutation within the claims. *See Angstadt*, 537 F.2d at 504.³ Instead, the specification need only sufficiently characterize the generic invention. *See Angstadt*, 537 F.2d at 504.

The Office provides no further evidence or reasoning to question the adequacy of the disclosure. For the reasons above, the specification adequately describes the cleavage motifs. The rejection thus must be withdrawn.

8. **Rejections under 35 U.S.C. § 102(b)**

[A] Okuno 2002b

Claim 1 stands rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Okuno 2002b. Applicants traverse the rejection as it applies to corresponding claim 36.

Okuno 2002b discloses the cleavage motif ELELYK↓RHHG. The Office alleges that the histidine at P3' (underlined above) is “a single basic amino acid . . . situated at any site in the amino acid sequence . . . from the P3' position to the P5' position,” as recited in claim 1. The claim element at issue does not occur in corresponding claim 36. Okuno 2002b does not disclose two or three consecutive basic amino acids situated in the amino acid sequence from a P10

³ Established judicial precedent does not require *any* reduction to practice to demonstrate possession. *See, e.g., Falkner v. Inglis*, 79 USPQ2d 1001, 1007 (Fed. Cir. 2006).

position to a P3 position. Okuno 2002b thus does not disclose each element of the presently claimed process, and the rejection must be withdrawn.

[B] Sugimura 1988b

Claims 1, 6, and 7 stand rejected as allegedly anticipated by Sugimura 1988b. Applicants traverse the rejection as it applies to corresponding claims 36, 45, and 46.

Sugimura discloses cleavage motifs in Table 2, including:

YGGFLR↓RIRPKLK	Dynorphin (1-13)
NLKALAALAK↓KIL	Mastoparan
GK↓RKRSQMLFRGRRASQ or	Human γ IFN
KRSQMLFRGR↓RASQ	

The Office alleges that the P3' Arg in the Dynorphin (1-13) cleavage motif (underlined above) is “a single basic amino acid . . . situated at any site in the amino acid sequence . . . from the P3' position to the P5' position,” as recited in claim 1. The claim element at issue does not occur in corresponding claim 36. Sugimura does not disclose two or three consecutive basic amino acids situated in the amino acid sequence from a P10 position to a P3 position. Sugimura thus does not disclose each element of the presently claimed process, and the rejection must be withdrawn.

[C] Okuno 2002a

Claims 1 and 34 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Okuno 2002a. Applicants traverse the rejection as it applies to corresponding claims 36 and 49.

The Office alleges that Okuno 2002a discloses several cleavage motifs, where the P3' position is the amino acid histidine. The Office alleges that histidine is a basic amino acid and that Okuno 2002a thus discloses “a single basic amino acid . . . situated at any site in the amino acid sequence . . . from the P3' position to the P5' position,” as recited in claim 1. The claim element at issue does not occur in corresponding claim 36. Okuno 2002a does not disclose two or three consecutive basic amino acids situated in the amino acid sequence from a P10 position to a P3 position. Okuno 2002a thus does not disclose each element of the presently claimed process, and the rejection must be withdrawn.

[D] Yabuta

Claims 1, 34, and 35 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Yabuta. Applicants traverse the rejection.

Yabuta discloses two cleavage motifs at FIG. 2a: YDAELRLYR↓RHHRW and IEDRLYR↓RHHRW. The Office alleges that histidine and arginine are both basic amino acids and that Yabuta thus discloses “two or three consecutive basic amino acids situated . . . from the P3' position to the P5' position” (underlined above), as recited in claim 1. The claim element at issue does not occur in corresponding claim 36. Yabuta does not disclose two or three consecutive basic amino acids situated in the amino acid sequence from a P10 position to a P3 position. Yabuta thus does not disclose each element of the presently claimed process, and the rejection must be withdrawn.

[E] Kramer

Claim 12 stands rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Kramer. Applicants traverse the rejection. Applicants traverse the rejection.

Kramer discloses the substrate Abz-Ala-Arg↓Arg-Ala-Dap(dnp)-Gly. *See* Part 10 of this response, above. Kramer does not teach that the P1' position is an amino acid other than arginine or lysine. Kramer thus does not disclose each element of the presently claimed process, and the rejection must be withdrawn.

9. Rejections under 35 U.S.C. § 103(a)

[A]

Claims 2 and 31-33 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Yamamoto in view of Dekker. Applicants traverse the rejection. The relevant corresponding new claims are claims 36-40 and 67-68.

Yamamoto allegedly discloses a fusion protein comprising a motilin sequence and a linker motif with P1/P1' positions Lys↓Arg. Dekker allegedly discloses the cleavage motif Arg↓Phe-Val at Table 2. Dekker discloses amino acid preferences at P1, P1', and P2' positions at p. 1697, 2nd col., under “*Library Screen*.”

Claim 36 recites in part cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position. A determination of *prima facie* obviousness requires that the proposed combination of references suggest or disclose each claim element. The combination of Yamamoto and Dekker, however, are silent regarding at least this claim element. Claims 36-40 depend from claim 36 and incorporate this claim element. *Prima facie* obviousness thus is not established, and the rejection must be withdrawn.

[B]

Claims 3, 8 and 9, stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Okuno (2002a), Okuno (2002b), or Sugimura (1988a) in view of Dekker and Okuno (2002b). Applicants traverse the rejection. The relevant corresponding new claims are 36, 42, and 66.

The disclosures of Okuno (2002a), Okuno (2002b), and Dekker are set forth above. Sugimura (1988a) is relied upon for a disclosure relevant to an acidic amino acid at position P3, which is recited in dependent claim 42. None of the references teaches or suggests at least cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claims 42 and 66, which depends directly or indirectly from claim 36. The rejection thus must be withdrawn.

[C]

Claims 4, 5 and 10 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Stumpe in view of Suzuki and Sugimura (1988a). Applicants traverse the rejection. The relevant corresponding new claims are 36, 43, and 46.

Stumpe discloses the degradation of peptide protamine by OmpT. Stumpe's substrates apparently do not comprise SEQ ID NO: 11, but Suzuki discloses a protamine substrate that does. The artisan allegedly would have been motivated by the combined teachings of Stumpe, Suzuki and Sugimura (1988a) to cleave SEQ ID NO: 11 with OmpT protease as follows:

Arg-Arg-Arg-Ala-Arg↓Arg
P5 -P4 -P3 -P2 -P1 -P1'

Stumpe discloses that the protamine substrate used in his experiments is a mixture of compounds. One of the major protamine compounds has the sequence MPRRRRSSSRPVRRRRRPRVSRRRRRRGRRRR. *See* Stumpe, p. 4004, 1st col., 2nd para. In the claimed process, two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position of the cleaved polypeptide. In Stumpe, however, the major disclosed component of the protamine substrate does not have a sequence that would read on this claim element, regardless of where the peptide was cleaved. Stumpe's substrate contains too many basic amino acids. Any sequence encompassing eight amino acids (P10 – P3) would include more than the recited two or three consecutive basic amino acids.

Suzuki's protamine substrate has the sequence:
PRRRTRRASRPVRRRRPRRVSRRRRAR↓RRR. The OmpT cleavage motif comprising SEQ ID NO: 11 is underlined. Suzuki's sequence, like Stumpe's, contains more than two or three basic amino acids situated in the amino acid sequence from a P10 position to a P3 position of the allegedly cleaved polypeptide. In fact it contains six (underlined):

Arg-Arg-Val-Ser-Arg-Arg-Arg-Arg-Ala-Arg↓Arg
P10-P9 -P8 -P7 -P6 -P5 -P4 -P3 -P2 -P1 -P1'

The Office is respectfully reminded that it is merely by chance that Suzuki's substrate contains SEQ ID NO: 11. None of the references teaches or suggests cleavage of P1-P1' having the claimed P10-P3 sequences. Furthermore, both above presented sequences contain many basic amino acids. There is no evidence which basic amino acid(s) are responsible for the P1-P1' cleavage.

Neither Stumpe, Suzuki, Sugimura (1988a) (*see* Part 9[B], *above*) alone, nor the combination thereof teach or suggest at least a process where two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position of the cleaved polypeptide. The rejection depends on the artisan taking a cleavage motif out of Suzuki's polypeptide that happens to be identical to SEQ ID NO: 11, but the combined

references do not motivate this modification. Instead, the rejection is based impermissibly on hindsight provided by Applicants' disclosure. It thus must be withdrawn.

[D]

Claim 34 stands rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), or Dekker in view of Grodberg. Applicants traverse the rejection. The corresponding new claims are claims 36 and 48.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker are set forth above. Grodberg is relied upon for her disclosure of a bacterial cell expressing OmpT. Claim 48 depends on claim 36. None of the cited references teaches or suggests at least cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claim 48, which depends from claim 36. The rejection thus must be withdrawn.

[E]

Claims 2, 31, 33, and 35 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker in view of Yabuta. Applicants traverse the rejection. The corresponding new claims are claims 36-38, 40-41, and 67.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Yabuta are set forth above. Claims 37-38 and 40-41 depend on claim 36. None of the cited references teaches or suggests at least cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claims 37-38 and 40-41, which depend from claim 36.

Claim 67 depends indirectly from claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid

variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of reference thus cannot establish *prima facie* obviousness of claim 67, which dependent from claim 49.

The rejection thus must be withdrawn.

[F]

Claims 12-14, 21, and 22 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker in view of Kramer. Applicants traverse the rejection. The corresponding new claims are claims 49, and 51-53, respectively.

Kramer discloses cleavage of a substrate by an OmpT D97A variant (*see* FIG. 2). Kramer uses the substrate Abz-Ala-Arg↓Arg-Ala-Dap(dnp)-Gly. *See* Part 10 of this response, above. Claim 49, however, is directed in part to a process for cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine. Kramer does not disclose a P1' position amino acid other than arginine or lysine.

Nor does Kramer suggest using an OmpT D97A variant to cleave alternate substrates. In fact, the D97A variant cleaved the Abz-Ala-Arg-Arg-Ala-Dap(dnp)-Gly substrate with only 6% efficiency, relative to wild-type OmpT. This relative inefficiency would not have prompted the skilled artisan to make other OmpT 97th amino acid variants, let alone test such variants with alternate substrates. The present specification for the first time teaches that an OmpT 97th amino acid variant efficiently cleaves particular substrates. The motivation to modify Kramer can only be provided by hindsight reasoning from Applicants' disclosure.

The disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker are set forth above. Some of the OmpT substrates disclosed in these references have an amino acid other than arginine or lysine at the P1' position. The Office has not explained, however, why the skilled artisan would have used an OmpT D97A variant showing only 6% of the wild-type activity to cleave any of the substrates disclosed in Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker. In particular, the Office has not explained what would have motivated the

artisan to select from all the disclosed substrates only those with a P1' position that is an amino acid other than arginine or lysine, as recited.

Here and elsewhere, the Office alleges that the artisan would have been motivated to combine the references by the general desire to cleave substrates with OmpT. The Office, however, has not explained why the artisan would have tried to cleave other substrates with D97A, when D97A cleaved the Arg↓Arg site with only 6% activity. If anything, Kramer teaches away from making 97th amino acid variants, because Kramer's only attempt to make such a variant produced poor results.

Accordingly, Kramer and Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker collectively do not motivate the Office's proposed combination. The rejection instead is based on hindsight afforded by Applicants' specification. Proper *prima facie* obviousness is not established, and the rejection thus should be withdrawn.

[G]

Claims 12-14, 21, and 22 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler. Applicants traverse the rejection. The corresponding claims are claims 49, and 51-53, respectively.

The deficiencies of the proposed combination of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer are discussed above. Metzler is a general teaching that amino acids are classified based on charge, hydrophobicity, and size. The Office proposes that it would have been obvious to substitute A97 with a glutamine residue, given the structural similarity between aspartic acid and glutamic acid. Even if this were true, and for the sake of argument only, Metzler does not motivate using an OmpT 97th amino acid variant with any of the substrates disclosed in Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Dekker. Metzler fails to cure the deficiencies of the combination of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer.

[H]

Claims 12-14, and 27-30 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler. Applicants traverse the rejection. The corresponding new claims are claims 49, 51, 50, and 63-65, respectively.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler are set forth above. Claims 51, 50, and 63-65 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of references thus cannot establish *prima facie* obviousness of claims 51, 50, and 63-65, which depend from claim 49.

Furthermore, Kramer merely speculates that P1' may cooperate with Asp⁹⁷ of the *E. coli* OmpT protease. See Kramer, lines 2 to 8, left col., page 429 (“**Assuming** that the substrate has an extended conformation and that the P1 site chain points toward Glu²⁷ and Asp²⁰⁸, the P1' chain **would be** located close to Asp⁹⁷.”) (emphasis added). There can be no reasonable expectation that replacement of the 97th aspartic acid with other amino acid would have changed substrate specificity, let alone the presently claimed amino acid substitution of Asp⁹⁷.

In view of above arguments, the rejection thus must be withdrawn.

[I]

Claims 12-14, 21, 22, 27, and 30 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura, Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Metzler. Applicants traverse the rejection. The corresponding new claims are claims 49, 51, 52, 53, 50, and 65, respectively.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler are set forth above. Claims 51, 52, 53, 50, and 65 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The

combination of references thus cannot establish *prima facie* obviousness of claims 51, 52, 53, 50, and 65, which depend from claim 49. The rejection thus must be withdrawn.

[J]

Claims 15-17 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura, Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Yabuta. Applicants traverse the rejection. The corresponding new claims are 49, 54, and 55, respectively.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Yabuta are set forth above. Claims 54 and 55 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of references thus cannot establish *prima facie* obviousness of claims 54 and 55, which depend from claim 49. The rejection thus must be withdrawn.

[K]

Claims 15-17 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler in view of Yabuta. Applicants traverse the rejection. The corresponding new claims are 49, 54, and 55, respectively.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, Metzler, and Yabuta are set forth above. Claims 54 and 55 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of references thus cannot establish *prima facie* obviousness of claims 54 and 55, which depend from claim 49. The rejection thus must be withdrawn.

[L]

Claims 18, 23, and 24 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler in view

of Dekker and Okuno (2002b). Applicants traverse the rejection. The corresponding new claims are 49, 57, and 58.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler are set forth above. Claims 57 and 58 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of references thus cannot establish *prima facie* obviousness of claims 57 and 58, which depend from claim 49. The rejection thus must be withdrawn.

[M]

Claims 19, 20, and 25 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer and Metzler in view of Stumpe and Suzuki. Applicants traverse the rejection. The corresponding new claims are 49, 59, 60, and 61.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, Metzler, Stumpe, and Suzuki are set forth above. Claims 60 and 61 depend on claim 49. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. The combination of references thus cannot establish *prima facie* obviousness of claims 60 and 61, which depend from claim 49. The rejection thus must be withdrawn.

[N]

Claim 34 stands rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, and Kramer in view of Grodberg. Applicants traverse the rejection. The corresponding new claims are 36 and 48.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Grodberg are set forth above. Claim 48 depends on claim 36. The combination of references does not teach or suggest at least cleaving a polypeptide with an *E. coli* OmpT

protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claim 48, which depends from claim 36. The rejection thus must be withdrawn.

[O]

Claim 34 stands rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler in view of Grodberg. Applicants traverse the rejection. The corresponding new claims are 36 and 48.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, Metzler, and Grodberg are set forth above. Claim 48 depends on claim 36. The combination of references does not teach or suggest at least cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claim 48, which depends from claim 36. The rejection thus must be withdrawn.

[P]/[Q]

Claim 35 stands rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Sugimura (1988a), Okuno (2002a), Okuno (2002b), and Kramer in view of Yabuta or alternately under Sugimura (1988a), Okuno (2002a), Okuno (2002b), Dekker, Kramer, and Metzler in view of Yabuta. Applicants traverse the rejection. The corresponding new claims are 41, 36, 37, 56, 54, and 49.

The relevant disclosures of Sugimura (1988a), Okuno (2002a), Okuno (2002b), Kramer, Metzler, and Yabuta are set forth above. Claim 41 depends on claims 36 and 37. Claim 56 depends on claims 49 and 54. The combination of references does not teach at least a process of cleaving a polypeptide with an *E. coli* OmpT protease 97th amino acid variant, where the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, as recited in claim 49. Nor the combination of references teach or suggest at least cleaving a polypeptide with an *E. coli* OmpT protease, wherein two or three consecutive basic amino acids

are situated in the amino acid sequence from a P10 position to a P3 position, as recited in claim 36. The combination of references thus cannot establish *prima facie* obviousness of claims 41 and 56, which depend indirectly from claims 36 and 49, respectively. The rejection thus must be withdrawn.

CONCLUSION

The claims are believed in condition for allowance, which is requested in light of the amendment and remarks above. Should the Office have any questions or comments regarding the amendments or response, please contact Applicants' undersigned representative. Please direct all correspondence to the below-listed address.

In the event that the Office believes that there are fees outstanding in the above-referenced matter and for purposes of maintaining pendency of the application, the Office is authorized to charge the outstanding fees to Deposit Account No. 50-0573. The Office is likewise authorized to credit any overpayment to the same Deposit Account Number.

Respectfully Submitted,

Date: June 16, 2010

By: 

Mercedes K. Meyer, Ph.D., Esq.
Registration No. 44,939

DRINKER BIDDLE & REATH LLP
Customer No. **55694**
1500 K Street, N.W., Suite 1100
Washington, D.C. 20005-1209
Tel. No.: (202) 842-8800
Fax No.: (202) 842-8465

Exhibit 1

Claims 1-35	Corresponding New Claims 36-68
<p>1. A process for cleaving a polypeptide comprising the steps of:</p> <p>(1) providing a polypeptide comprising arginine or lysine at the P1 position of a desired cleavage site, an amino acid other than aspartic acid, glutamic acid or proline at the P1' position, and a single basic amino acid or two or three consecutive basic amino acids situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position, wherein, if there is only a single basic amino acid situated in the amino acid sequence from the P10 position to the P3 position, the single basic amino acid is situated at a position other than the P6 or the P4 position; and</p> <p>(2) cleaving the polypeptide with <i>E. coli</i> OmpT protease.</p>	<p>36. A process for cleaving a polypeptide comprising cleaving the polypeptide with an <i>E. coli</i> OmpT protease,</p> <p>wherein the polypeptide comprises a cleavage site that is a peptide bond between a P1 position and a P1' position,</p> <p>wherein the P1 position is arginine or lysine;</p> <p>wherein the P1' position is an amino acid other than aspartic acid, glutamic acid or proline; and</p> <p>wherein two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position.</p>
<p>2. The process according to claim 1, wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the protecting peptide is the P1' position, and wherein the fusion protein is produced by expressing a gene encoding the fusion protein in host cells in the step (1), and the fusion protein is cleaved with <i>E. coli</i> OmpT protease so as to liberate the target peptide in the step (2).</p>	<p>37. The process according to claim 36, wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide,</p> <p>wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the target peptide is the P1' position,</p> <p>wherein the fusion protein is produced by expressing a gene encoding the fusion protein in a host cell, and</p> <p>wherein cleavage of the fusion protein liberates the target peptide.</p>
<p>3. The process of claim 1 wherein, if a site which is not desired to be cleaved by <i>E. coli</i> OmpT protease is present in the polypeptide, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.</p>	<p>42. The process of claim 36, wherein, if a site which is not desired to be cleaved by the <i>E. coli</i> OmpT protease is present in the polypeptide, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position.</p>

Claims 1-35	Corresponding New Claims 36-68
4. The process of claim 1, wherein two or three consecutive basic amino acids are situated between the P10 and P3 positions of the desired cleavage site in the polypeptide.	
5. The process of claim 4, , wherein three consecutive basic amino acids are situated between the P5 and P3 positions of the desired cleavage site in the polypeptide.	43. The process of claim 36, wherein three consecutive basic amino acids are situated between the P5 and P3 positions in the polypeptide.
6. The process of claim 1, wherein the basic amino acids are arginine and/or lysine.	44. The process of claim 36, wherein the basic amino acids are arginine and/or lysine.
7. The process of claim 6, wherein the basic amino acids are arginine.	45. The process of claim 44, wherein the basic amino acids are arginine.
8. A process for cleaving a polypeptide comprising cleaving a polypeptide at a desired cleavage site with <i>E. coli</i> OmpT protease, or producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein, wherein, if a site which is not desired to be cleaved by <i>E. coli</i> OmpT protease is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.	
9. The process of claim 8, wherein the acidic amino acid is aspartic acid.	66. The process of claim 42, wherein the acidic amino acid is aspartic acid.
10. The process of claim 1, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).	46. The process of claim 36, wherein the amino acid sequence from the P5 to P1 positions in the polypeptide is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).
11. The process of claim 1, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).	47. The process of claim 36, wherein the amino acid sequence from the P7 to P1 positions in the polypeptide is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).

Claims 1-35	Corresponding New Claims 36-68
<p>12. A process for cleaving a polypeptide comprising cleaving a desired cleavage site of a polypeptide or a fusion protein using an <i>E. coli</i> OmpT protease 97th amino acid variant to produce a target peptide, wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.</p>	<p>49. A process for cleaving a polypeptide comprising cleaving the polypeptide with an <i>E. coli</i> OmpT protease 97th amino acid variant, wherein the 97th amino acid from the N-terminus of the <i>E. coli</i> OmpT protease 97th amino acid variant is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, wherein the polypeptide comprises a cleavage site that is a peptide bond between a P1 position and a P1' position, and wherein the P1 position is arginine or lysine and the P1' position is an amino acid other than arginine or lysine.</p>
<p>13. The process of claim 12, wherein the P1 position of the desired cleavage site in the polypeptide is arginine or lysine and the P1' position is an amino acid other than arginine or lysine.</p>	
<p>14. The process of claim 13, wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position.</p>	<p>51. The process of claim 49, wherein a single basic amino acid or two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position.</p>

Claims 1-35	Corresponding New Claims 36-68
<p>15. A method for producing a target peptide, comprising transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a target peptide fused with a protecting peptide via a desired cleavage site that can be cleaved by an <i>E. coli</i> OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the <i>E. coli</i> OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.</p>	<p>54. The process of claim 49, wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide, wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the target peptide is the P1' position, wherein the fusion protein is produced by expressing a gene encoding the fusion protein in a host cell, and wherein cleavage of the fusion protein liberates the target peptide.</p>
<p>16. A method for producing a target peptide comprising transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site that can be cleaved by an <i>E. coli</i> OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the <i>E. coli</i> OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.</p>	

Claims 1-35	Corresponding New Claims 36-68
<p>17. The method of claim 16, wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position at a desired cleavage site of a fusion protein.</p>	<p>55. The method of claim 54, wherein a single basic amino acid or two or three consecutive basic amino acids are situated in the amino acid sequence from a P10 position to a P3 position.</p>
<p>18. The process of claim 12 wherein, if a site which is not desired to be cleaved by the <i>E. coli</i> OmpT protease 97th amino acid variant is present in the polypeptide or the fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.</p>	<p>57. The process of claim 49, wherein, if a site which is not desired to be cleaved by the <i>E. coli</i> OmpT protease 97th amino acid variant is present in the polypeptide, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position.</p>
<p>19. The process of claim 12, wherein two or three consecutive basic amino acids are situated between the P10 and P3 positions of the desired cleavage site in the polypeptide or the fusion protein.</p>	<p>59. The process of claim 49, wherein two or three consecutive basic amino acids are situated between P10 and P3 positions in the polypeptide.</p>
<p>20. The process of claim 19, wherein three consecutive basic amino acids are situated between the P5 and P3 positions of the desired cleavage site in the polypeptide or the fusion protein.</p>	<p>60. The process of claim 59, wherein three consecutive basic amino acids are situated between P5 and P3 positions in the polypeptide.</p>
<p>21. The process of claim 14, wherein the basic amino acids are arginine and/or lysine.</p>	<p>52. The process of claim 51, wherein the basic amino acids are arginine and/or lysine.</p>
<p>22. The process of claim 21, wherein the basic amino acids are arginine.</p>	<p>53. The process of claim 52, wherein the basic amino acids are arginine.</p>

Claims 1-35	Corresponding New Claims 36-68
<p>23. A process for cleaving a polypeptide comprising cleaving a polypeptide at a desired cleavage site with an <i>E. coli</i> OmpT protease 97th amino acid variant, or producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein, wherein if a site which is not desired to be cleaved by the <i>E. coli</i> OmpT protease 97th amino acid variant is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.</p>	
<p>24. The process of claim 18, wherein the acidic amino acid is aspartic acid.</p>	<p>58. The process of claim 57, wherein the acidic amino acid is aspartic acid.</p>
<p>25. The process of claim 12, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or the fusion protein is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).</p>	<p>61. The process of claim 49, wherein the amino acid sequence from P5 to P1 positions in the polypeptide is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).</p>
<p>26. The process of claim 12, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or the fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).</p>	<p>62. The process of claim 49, wherein the amino acid sequence from P7 to P1 positions in the polypeptide is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).</p>
<p>27. The process of claim 12, wherein the 97th amino acid from the N-terminus of the <i>E. coli</i> OmpT protease is leucine, methionine or histidine.</p>	<p>50. The process of claim 49, wherein the 97th amino acid from the N-terminus of the <i>E. coli</i> OmpT protease variant is leucine, methionine or histidine.</p>
<p>28. The process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is serine or alanine, and the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant used is leucine.</p>	<p>63. The process of claim 49, wherein the P1' position is serine or alanine, and wherein the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant is leucine.</p>

Claims 1-35	Corresponding New Claims 36-68
<p>29. The process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is phenylalanine, alanine, serine, cysteine or tyrosine, and the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant used is methionine.</p>	<p>64. The process of claim 49, wherein the P1' position is phenylalanine, alanine, serine, cysteine or tyrosine, and wherein the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant is methionine.</p>
<p>30. The process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is alanine, valine, isoleucine, methionine, serine, threonine, cysteine or asparagine, and the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant used is histidine.</p>	<p>65. The process of claim 49, wherein the P1' position is alanine, valine, isoleucine, methionine, serine, threonine, cysteine or asparagine, and wherein the 97th amino acid of the <i>E. coli</i> OmpT protease 97th amino acid variant is histidine.</p>
<p>31. The process of claim 2, wherein the target peptide is a peptide composed of between 22 and 45 amino acid residues.</p>	<p>38. The process of claim 37, wherein the target peptide is composed of between 22 and 45 amino acid residues.</p> <p>67. The process of claim 54, wherein the target peptide is composed of between 22 and 45 amino acid residues.</p>
<p>32. The process of claim 31, wherein the target peptide is adrenocorticotrophic hormone (1-24), motilin or calcitonin precursor.</p>	<p>39. The process of claim 38, wherein the target peptide is adrenocorticotrophic hormone (1-24), motilin or calcitonin precursor.</p> <p>68. The process of claim 67, wherein the target peptide is adrenocorticotrophic hormone (1-24), motilin or calcitonin precursor.</p>
<p>33. The process of claim 2, wherein the host cells are <i>E. coli</i>.</p>	<p>40. The process of claim 37, wherein the host cell is <i>E. coli</i>.</p>

Claims 1-35	Corresponding New Claims 36-68
<p>34. The process of either claim 1 or claim 12, wherein the polypeptide is cleaved by using, as the cleaving protease, bacterial cells expressing a gene coding for <i>E. coli</i> OmpT protease or an <i>E. coli</i> OmpT protease 97th amino acid variant, and wherein the 97th amino acid from the N-terminus of <i>E. coli</i> OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.</p>	<p>48. The process of claim 36, wherein the <i>E. coli</i> OmpT protease is an <i>E. coli</i> OmpT protease 97th amino acid variant, wherein the 97th amino acid from the N-terminus of <i>E. coli</i> OmpT protease variant is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.</p>
<p>35. The process of either claim 1 or claim 12, wherein a gene coding for <i>E. coli</i> OmpT protease or an <i>E. coli</i> OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of <i>E. coli</i> OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, is co-expressed with a gene coding for a polypeptide whose cleavage by said protease is desired.</p>	<p>41. The process of claim 37, wherein the <i>E. coli</i> OmpT protease is produced by expressing a gene encoding the <i>E. coli</i> OmpT protease in said host cell.</p> <p>56. The process of claim 54, wherein the <i>E. coli</i> OmpT protease 97th amino acid variant is produced by expressing a gene encoding the <i>E. coli</i> OmpT protease 97th amino acid variant in said host cell.</p>

Attorney Docket No.: 47259-5001-00 US (223490)
Application No.: 10/573,821
Response to Office Action mailed: February 16, 2010
Response filed: June 16, 2010
Page 40

Exhibit 2

Excerpt from GenBank Accession No. YP_444072.1

Protein

Translations of Life

Display Settings: GenPept

outer membrane protease [Escherichia coli]

NCBI Reference Sequence: YP_444072.1

Comment Features Sequence

LOCUS YP_444072 317 aa linear BCT 29-MAR-2010
 DEFINITION outer membrane protease [Escherichia coli].
 ACCESSION YP_444072
 VERSION YP_444072.1 GI:84060870
 DBLINK Project:16260
 DBSOURCE REFSEQ: accession NC_007675.1
 KEYWORDS .
 SOURCE Escherichia coli
 ORGANISM Escherichia coli
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
 Enterobacteriaceae; Escherichia.

REFERENCE 1 (residues 1 to 317)
 AUTHORS Skyberg,J.A., Johnson,T.J., Johnson,J.R., Clabots,C., Logue,C.M.
 and Nolan,L.K.
 TITLE Acquisition of avian pathogenic Escherichia coli plasmids by a
 commensal E. coli isolate enhances its abilities to kill chicken
 embryos, grow in human urine, and colonize the murine kidney
 JOURNAL Infect. Immun. 74 (11), 6287-6292 (2006)
 PUBMED [16954398](#)

REFERENCE 2 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Putative virulence region of a ColV plasmid from an avian
 pathogenic Escherichia coli (APEC)
 JOURNAL Unpublished

REFERENCE 3 (residues 1 to 317)
 CONSRTM NCBI Genome Project
 TITLE Direct Submission
 JOURNAL Submitted (29-DEC-2005) National Center for Biotechnology
 Information, NIH, Bethesda, MD 20894, USA

REFERENCE 4 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Direct Submission
 JOURNAL Submitted (14-DEC-2005) Veterinary Microbiology and Preventive
 Medicine, Iowa State University, 1802 Elwood Drive, VMRI 2, Ames,
 IA 50011, USA

REMARK Sequence update by submitter

REFERENCE 5 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Direct Submission
 JOURNAL Submitted (11-AUG-2005) Veterinary Microbiology and Preventive
 Medicine, Iowa State University, 1802 Elwood Drive, VMRI 2, Ames,
 IA 50011, USA

REMARK Sequence update by submitter

REFERENCE 6 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Direct Submission
 JOURNAL Submitted (28-FEB-2005) Veterinary Microbiology and Preventive
 Medicine, Iowa State University, 1802 Elwood Drive, VMRI 2, Ames,
 IA 50011, USA

REMARK Sequence update by submitter

REFERENCE 7 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Direct Submission
 JOURNAL Submitted (20-MAY-2004) Veterinary Microbiology and Preventive
 Medicine, Iowa State University, 1802 Elwood Drive, VMRI 2, Ames,
 IA 50011, USA

REMARK Sequence update by submitter

REFERENCE 8 (residues 1 to 317)
 AUTHORS Johnson,T.J. and Nolan,L.K.
 TITLE Direct Submission
 JOURNAL Submitted (09-FEB-2004) Veterinary Microbiology and Preventive
 Medicine, Iowa State University, 1802 Elwood Drive, VMRI 2, Ames,
 IA 50011, USA

COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The
 reference sequence was derived from [ABA54754](#).
 Method: conceptual translation.

FEATURES Location/Qualifiers

```
source      1..317
            /organism="Escherichia coli"
            /strain="A2363"
            /db_xref="taxon:562"
            /clone="Contig 42"
            /plasmid="pAPEC-O2-ColV"
            /note="avian pathogenic strain"
Protein     1..317
            /product="outer membrane protease"
            /EC_number="3.4.23.49"
            /name="outer membrane protein; protease precursor; similar
            to SwissProt Accession Number P58603"
            /calculated_mol_wt=35503
Region      27..317
            /region_name="OmpT"
            /note="OmpT family; cl01886"
            /db_xref="CDD:174682"
CDS         1..317
            /gene="ompT"
            /locus_tag="O2ColV26"
            /coded_by="complement(NC_007675.1:114321..115274)"
            /note="outer membrane protease; involved in virulence in
            many organisms; OmpT; IcsP; SopA; Pla; PgtE; ompT; in
            Escherichia coli OmpT can degrade antimicrobial peptides;
            in Yersinia Pla activates plasminogen during infection; in
            Shigella flexneria SopA cleaves the autotransporter IcsA"
            /transl_table=11
            /db_xref="GeneID:3853531"
ORIGIN
1 mylkilatal sapvafaala sdtglsftpe kisteidfgt lsgkakervy lpeekgrkas
61 qldwkysnap ivkgafnwdl lprsvgasg wttlagrggn mvdrdwldts npgtwtdesk
121 hpntrlfnan efdlnikgwl lngpdyqlgl magyqenrys ftakggsyiy sseggfrdet
181 gsfpdgerai gykqhfkmpy igltgnyryd sfefggsfky sgwvkasdnd ehynpekrit
241 yrsdvnnqny ysvslhagyy itpaakvyve gtwnrtnkk gdtslysrnl nisdhtknga
301 giesynfmet aglkyyf
//
```

Exhibit 3

Abbreviations Used for Cited References

Dekker *et al.*, 40 BIOCHEMISTRY 1694 (2001) (“Dekker”)
Grodberg *et al.*, 170 J. BACTERIAL. 1245 (1988) (“Grodberg”)
Kramer *et al.*, 505 FEBS LETTERS 426 (2001) (“Kramer”)
Metzler, BIOCHEMISTRY, 2nd ed., Harcourt / Academic Press (2001) (“Metzler”)
Okuno *et al.*, 66 BIOSCI. BIOTECHNOL. BIOCHEM. 127 (2002) (“Okuno 2002a”)
Okuno *et al.*, 36 BIOTECHNOL. APPL. BIOCHEM. 77 (2002) (“Okuno 2002b”)
Stumpe *et al.*, 180 J. BACTERIOL. 4002 (1998) (“Stumpe”)
Sugimura *et al.*, 170 J. BACTERIOL. 3650 (1988) (“Sugimura 1988a”)
Sugimura *et al.*, 170 J. BACTERIOL. 5625 (1988) (“Sugimura 1988b”)
Suzuki *et al.*, 72 J. BIOCHEM. 1419 (1972) (“Suzuki”)
Yabuta *et al.*, 44 APPL. MICROBIOL. BIOTECHNOL. 118 (1995) (“Yabuta”)
U.S. Patent No. 5,506,120 (“Yamamoto”)